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Behavioral Changes in Adult Rats After Prenatal Exposures to Complex, Weak Magnetic Fields

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More than 100 adult male and female albino Wistar rats that had been exposed during their entire prenatal development to one of two patterns of magnetic fields and one of four intensities (reference: 5 to 20 nT; low: 30 to 50 nT; medium: 90 to 580 nT; high: 590 nT to 1.2 microT) were tested for their capacity for two forms of classical conditioning. The rats exposed for 10 sec every 50 sec to a field composed of successive 200 msec sequences of several different patterns known to produce physiological effects exhibited significantly more intense conditioned fear and taste aversion than those exposed continuously to a single frequency-modulated pattern. The behavioral differences, relative to the reference group (''controls''), were greatest for rats exposed to the 30 to 50 nT or 90 to 580 nT (low to medium intensities) for both patterns of fields. These results suggest that prenatal exposure to physiologically-patterned magnetic fields within a specific ''window'' of intensities that overlap with values found in many human habitats may produce long-term changes in behaviors.

Keywords Magnetic fields; Development; Rat; Conditioned fear; Conditioned taste aversion.

Introduction

Within the last 20 years most of the research concerning the effects of prenatal exposures to extremely low-frequency magnetic fields has focused upon 60 Hz or 50 Hz sine-waves within the mT range, the dominant power frequencies of western civilization (Brent, 1999). Pulsed fields, which have been shown to modulate gene expression in myocardial cells (Ventura et al., 2000), may be more biologically effective than more temporally symmetrical sine-waves (Zusman et al., 1990). These fields, when generated within the home environment, could be contributing factors for the induction of oncogenes Cfos ab1 and integrin alpha 5 beta 1 (Omura and Bro, 1993).

St-Pierre et al. (1998) suggested that emergent temporal forms similar to endogenous patterns generated within the brain could be created by the simultaneous exposure to multiple sources of man-made and natural magnetic fields. Rats with experimentally induced epilepsy displayed significant increased intragroup aggression

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as well as aberrant sexual behavior when specific physiologically patterned magnetic fields within the nanoTesla to microTesla range (similar to modern human habitats) were generated through their living space. St-Pierre and her colleagues hypothesized that as the numbers of sources of electromagnetic fields (from cell phones to computers) increase, the complexity of the combined magnetic fields and the probability of their potential "structural similarity" to endogenous neuroelectrical patterns would approach unity. As a result, very weak magnetic fields might alter the developing brain in a manner sufficient to influence fundamental adult behaviors such as the capacity for classical conditioning.

Complexity of the temporal form of the applied magnetic fields might allow significant biological effects at intensities much less than sine wave shapes. McKay et al. (2000), Persinger (2003), and Stewart and Persinger (2000) showed that while sine-wave fields did *not* affect contextual fear conditioning, very specific patterns of magnetic fields when applied for 30 min to adult rats affected specific behaviors strongly correlated with similar patterns endogenously generated within different brain structures. The effective intensities were between 20 and 2,000 nT, or .2–20 mG, well within the range of routine human exposure. These laboratory results strongly corroborate the myriad of correlations between behavioral alterations in several species and complex patterns of weak magnetic fields generated by geophysical processes (Cherry, 2002; Michon and Persinger, 1997).

In the present study, we exposed rats during their entire prenatal development to one of two types of complex, weak magnetic fields whose structures were patterned after physiologically relevant activity. The first was a frequency-modulated field that has been shown to induce aggression (St-Pierre et al., 1998) and seizures in epileptic rats (Persinger and Bellanger-Chellew, 1999) and analgesia in snails (Thomas et al., 1997). The second pattern was custom-constructed, from both theory (Wei, 1969), known effects of pulse-timing on the development of long-term potentiation in hippocampal slices (Rose et al., 1988), and a general appreciation of physiological patterns, to affect development. It was called the complex sequence (CS) field because it contained a series of different patterns that have been shown to induce qualitative morphological alterations in the hippocampal formation (St-Pierre and Persinger, 2003). Rats that were exposed during their entire prenatal development to this complex temporally structured magnetic field showed qualitatively conspicuous changes in open field behavior (Persinger et al., 2001) and much less proficient maze learning (McKay et al., 2003).

The purpose of the present study was to discern if exposures to a range of different intensities of the two shapes of magnetic fields during prenatal development would produce long-term changes in other behaviors. Conditioned taste aversion and conditioned fear were selected because of their ease of measurement and their prevalence over family, genus, and species.

Methods

Subjects

Conditioned Taste Aversion. A total of 36 male and 37 female rats, approximately 5–6 months of age, that had been exposed to 1 of the 2 patterns and 1 of the 4 intensities of each pattern during their prenatal development were selected as

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subjects. Not more than two rats from a given intensity for a given pattern from a given block were selected in order to minimize effects.

Conditioned Fear. A total of 43 male and 39 female rats, approximately 5–6 months of age, were selected as subjects. About half the numbers of rats had been exposed prenatally to the frequency-modulated field while the others had been exposed to the complex sequence field. More or less equal numbers within each pattern had been exposed to one of the four intensities.

General Procedure

All experiments had been approved by the university's Animal Care Committee (ACC) according to the guidelines of the Canadian Council on Animal Care. In 6 separate blocks of experiments 48 female, 90–100 day-old Wistar albino rats and 24 male, 90–120 day-old male Wistar rats that had been obtained from Charles River Breeders were housed in 1 of 4 equally sized compartments within the same plastic cage whose dimensions were 140 cm by 33 cm by 33 cm. There were two females and one male per compartment. At each end of the cage was a magnetic coil. The two coils were identical and were created by wrapping plastic cartons (milk crates) with 305 m of 30 AWG (Belden #9978) wire in a single layer (18 cm wide). The crates had been obtained from FREM Corporation (no 701-4) and were 38 cm × 33 cm × 27 cm. The circuitry was designed so that only one coil would be activated for any given block of experiments.

Three of the blocks involved the employment of the complex sequenced pattern. These sequences were presented for 10 s every 50 s, i.e., 10 s on, 40 s off. The other three blocks employed the continuous presentation of the frequency-modulated pattern where the point durations were 3 ms (St-Pierre et al., 2008). For two of the blocks for each pattern the activated coil was at one end of the cage while for the other block the other coil was activated. This allowed control for possible unknown artifacts associated with any of the four compartments. Because there was no statistically significant difference in the compartmental effects as a function of distance from either of the activated coils, the data were analyzed as a function of the distance from the activated coil.

The specific patterns of the magnetic fields were generated by converting columns of numbers, each containing a number between 0 and 255 into voltages by an XT personal computer. Other details have been published previously (Persinger et al., 2001; St-Pierre and Persinger, 2003). The point durations (the time the voltage specified by a given number was maintained) were 3 ms for the frequency-modulated pattern and 1 ms for the complex sequence pattern. The rise time between successive points was 100 μs.

The two patterns, frequency-modulated and complex-sequence, were initiated upon introduction of the triad of rats into the compartments and continued until the pups were delivered. To avoid competition between the two mothers, the second female was added to the compartments about five days after the first male and female were introduced. The males were removed 10 d after the beginning of the experiment (5 d after the introduction of the second female). We assumed 5 d were sufficient for the occurrence for one oestrus required for impregnation. During the experiment, Purina rat chow and water were available ad libitum. The light dark cycle was 12:12 with the scotophase beginning at 2,000 h. Corncob bedding was removed and replaced once every 2–3 d, during which time the rats were outside of the field for about 4 min.

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The ranges of intensities of the complex magnetic fields within the four compartments as a function of distance from the activated coil decreased in a nonlinear fashion. The four successive ranges, from either activated coil, were 590–1150 nT (11.5 mG), 90–180 nT, 30–50 nT, and 5–20 nT, respectively, within the compartments. The light intensity from overhead fluorescent lamps was approximately 500 lux at the floor of the exposure cages. Care was taken to insure that extraneous sources of power-frequency magnetic fields, such as those from clocks and computers, were distant from the cages, or that stray magnetic fields through the walls from other experimenters within the colony were not influential.

When the litters were born they were removed within 24 h and culled to 4 male and 4 female pups. The litters and their mothers were placed in separate cages in another room. A total of 15 (of 24) were delivered during the three blocks of exposure to the frequency-modulated field and a total of 18 litters (of 24) were delivered during the exposure to the complex sequence magnetic field. With the exception of the highest intensity groups exposed to the frequency-modulated pattern ($n = 3$ litters), there were 4–5 litters per intensity per condition.

Conditioned Taste Aversion. In six separate blocks of experiments over a one-year period, singly housed rats were habituated to 23.5 h water deprivation for 5 successive days. During this time water was available through graduated 100 cc bottles for 20 min each day during the mid-light phase. On the training day the rats received 100 cc of 10% sucrose solution rather than water for 20 min. Within 30 min after this period each rat was injected intraperitoneally with 10 cc/kg of 0.15 M Lithium Chloride in distilled water, a procedure routinely employed in our laboratory (Persinger and Fiss, 1978).

After two more days of an option to drink water only, they were given free access to 10% sucrose solution. The amounts (cc) of sucrose each rat consumed on the training day (first day) and the test day were measured. The CTA ratio was calculated by dividing the amount of sucrose consumed on the test day compared to the pairing or training day.

Conditioned Fear. On the training day the rat was placed in a 28 cm × 20 cm × 20 cm modified Plexiglas and aluminium operant chamber. The raised floor of the chamber consisted of 18 steel rods spaced 1.5 cm apart. Each animal was placed in the conditioned chamber and received four unsignaled foot-shocks (1-s, 0.5 mA) at 60 s intervals after an initial 2 min habituation period. Twenty-four hours later each rat was returned to the conditioning room. The rat's behaviors were scored for the presence of absence of freezing (not moving) via a time sampling procedure for 1 s every 8 s through the 8 min observation period. The total number of freezing observations (maximum = 60) was recorded for each rat.

Statistical Analyses. Three-way analyses of variance were completed as a function of pattern, intensity, and sex. Post hoc differences were set at $p < 0.05$ (Tukey). All analyses involved SPSS software on a VAX 4000 computer.

Results

Conditioned Taste Aversion. The rats that had been exposed to the complex-sequenced field consumed significantly [$F(1,62) = 16.35$, $p < 0.001$] less sucrose water during the pairing day ($M = 16.7$, $SD = 4.4$) than those that had been

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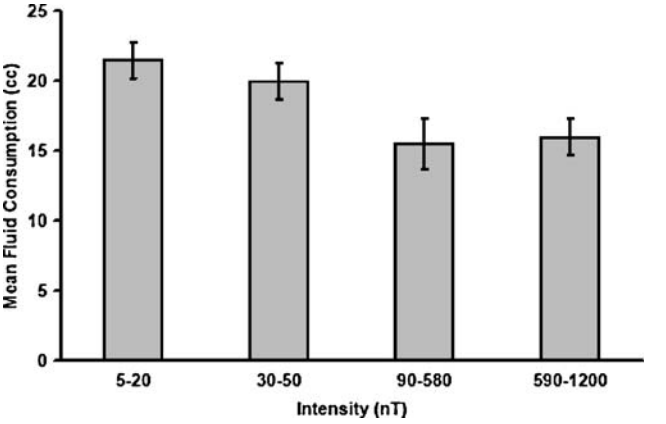

Figure 1. Means and S.E.M.s (vertical bars) for the volume of 10% sucrose solution consumed by all rats that had been exposed prenatally to one of the four intensities regardless of sex and type of magnetic field. There were between 14 and 18 rats per group.

exposed to the frequency-modulated pattern (M = 20.5, SD = 6.2). This treatment explained about 11% of the variance. There was a statistically significant [$F(3,62) = 2.81$, $p < 0.05$] difference as a function of intensity of the fields, regardless of the sex of the rats or the patterns to which they had been exposed. The means and standard deviations for the amount of sucrose consumed for these four groups of rats are shown in Figure 1.

Polynomial analyses clearly showed that the relationship between the amount of sugar water consumed the first time the rats were given this substance and the intensity of the prenatal field was linear [$F(1,62) = 8.49$, $p < 0.01$] and did not deviate from a linear function [$F(2,62) = 1.35$, $p < 0.05$]. Post hoc analyses indicated that the rats that had been exposed to the medium intensity magnetic field drank significantly less sucrose water during their first exposure compared to those rats that had been exposed to the low-intensity fields. Additional polynomial analyses clearly indicated that the relationship between intensity of the prenatal field, regardless of pattern and sex of the animal, was linear.

Three-way analysis of variance for the amount of sugar water consumed on the test day, three days following the pairing between the first exposure to sugar water and the injection of lithium, indicated only a significant difference between rats that had been exposed to the different patterns [$F(1,57) = 39.34$, $p < 0.001$; eta = .58]. The means and the standard deviations (in parentheses) for the amounts of sucrose consumed by the rats that had been exposed parentally to the complex-sequenced and frequency-modulated fields were 4.4 (2.8) cc and 9.7 (4.2) cc, respectively.

The rats that had been exposed prenatally to the complex-sequenced field displayed significantly [$F(1,57) = 17.20$, $p < 0.001$] *greater* taste aversion than the rats that had been exposed to the frequency-modulated field. The eta value for this effect was .36 and explained about 14% of the variance in the CTA scores. The mean and standard deviation for CTA scores for the rats that had been exposed to the complex-sequenced pattern was .26 and .17, while these values for the rats that had been exposed to the frequency-modulated field was .58 and .55, respectively. The rats that had been exposed to the complex-sequenced field showed greater bait

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shyness (CTA) while the rats that had been exposed to the frequency modulated field exhibited significantly more variance (Bartlett-Box $F = 38.53$, $p < 0.001$).

The intensity dependence for the CTA scores approached statistical significance. The test for homogeneity of variance (Bartlett Box $F = 16.19$, $p < 0.01$) was statistically significant and was due to the greater variability of scores within the rats that had been exposed to the medium intensity fields. When this discrepancy was accommodated by removing the two extreme scores, the means and standard deviations for reference, low intensity, medium intensity, and high intensity groups were .35 (.11), .46 (.27), .50 (.30), and .44 (.36), respectively.

Post hoc analysis indicated that the rats that had been exposed to low intensity (30–50 nT) field showed less aversion than did the rats exposed to the reference intensity (5–20 nT). The variability as defined by the standard deviation within the low, medium, and high intensity groups was more than twice that of the variability of the reference group. The sex effect was still significant although the effect size (4%) was markedly reduced. Post hoc analyses also indicated that the source of the significant interaction between sex and intensity was due to the weaker aversion developed by the females that had been exposed to the low intensity and particularly the medium intensity fields compared to the male rats that had been exposed to these intensities, regardless of pattern. These data are shown in Figure 2. There was no significant difference between the CTA scores for the males and female rats that had been exposed to either the reference or the high-intensity fields.

Contextual Fear. The results of the three-way analysis of variance for the conditioned fear responses demonstrated that the rats that had been exposed to the complex-sequenced pattern displayed significantly [$F(1,66) = 17.20$, $p < 0.001$] more "freezing" behavior than did the rats that had been exposed to the frequency-modulated pattern. The effects of exposure to either the frequency-modulated or complex-sequence field accommodated about 16% of the variance in this measure of immobility.

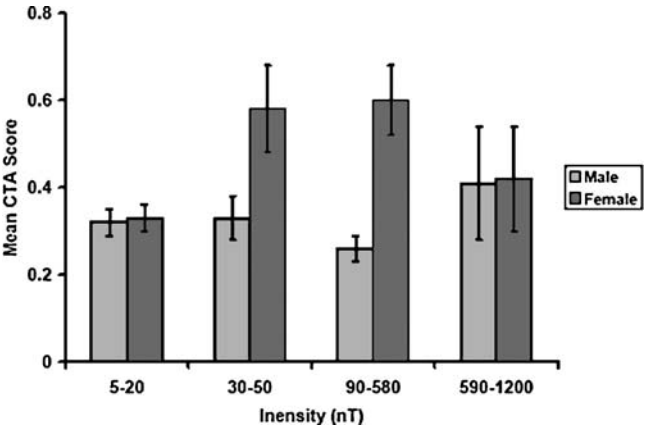

Figure 2. Means and S.E.M.s (vertical bars) of ratios for Conditioned Taste Aversion (CTA) for males and females that had been exposed to one of the four intensities regardless of field pattern. There were between 7 and 9 rats per group.

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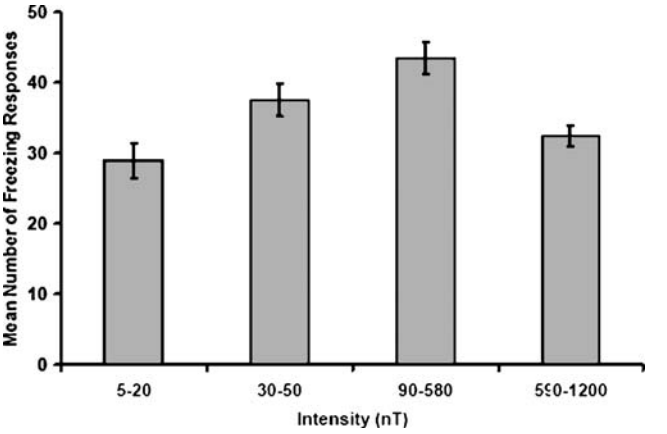

Figure 3. Means and S.E.M.s (vertical bars) for the numbers of times (maximum 60) the rat was immobile ("freezing") within the context during an 8 min period after one session of negative pairing with the context. Higher scores are indications of stronger conditioned fear.

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Post hoc analysis of the significant [$F(3,66) = 2.84$, $p < 0.05$] intensity effect (eta = .36) indicated that the rats that had been exposed to the medium (especially) and low intensity, regardless of pattern, exhibited more freezing behavior than the rats that had been exposed to the reference or to the highest intensity magnetic fields (Figure 3). As indicated by the interaction between sex and intensity that approached statistical significance, the greatest freezing behavior for males occurred with those that had been exposed to the low-intensity field while for the females the greatest amount occurred with those that had been exposed to the medium intensity fields.

The statistically significant [$F(1,66) = 4.63$, $p < 0.01$] interaction between sex and pattern was due to the greater discrepancy (mean, M = number of observations of immobility) between the males that had been exposed prenatally to the complex sequence fields (M = 50) compared to the frequency modulated field (M = 29) and the females that had been exposed to the complex-sequenced fields (M = 40) compared to the females that had been exposed to the frequency-modulated fields (M = 33).

Body Weights. The means and standard deviations for the body weights for the male rats that had been exposed to the four intensities (control to high) of the frequency-modulated fields were 506 g (85), 515 g (65), 484 g (67), and 477 g (40); the values for the males exposed to the complex-sequenced field were 591 g (81), 697 g (143), 712 g (168), and 651 g (129), respectively. Two-way analysis of variance as a function of pattern and intensity (all dfs = 1,41) showed that the males exposed to the complex-sequenced field during prenatal development were significantly heavier ($F = 28.61$, $p < 0.001$) than those exposed to the frequency-modulated fields.

The means and standard deviations for the body weight of female rats exposed to the frequency-modulated fields were 300 g (25), 290 g (19), 323 g (45), and 310 g (31); these values for the complex-sequenced field were 326 g (64), 374 g (75), 401 g (79), and 389 g (84), respectively. There were no statistically significant differences

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between the females with respect to the patterns or intensities to which they had been exposed (all dfs = 1,48).

Discussion

If the conceptual basis of our approach is valid then different temporal patterns of weak magnetic fields, like the different molecular structures of different pharmacological agents, should produce different and specific effects within biological systems. Like different molecular structures whose maximum efficacy: (1) varies with their concentrations; and (2) may exhibit a linear or nonlinear dose-relationship depending upon the receptor subtype, different patterns of complex magnetic fields should exert different effects upon some behaviors. Different effects from different patterns might not be evident for other behaviors mediated by other neural processes. Instead, a number of different patterns might evoke intensity-dependent changes upon a behavior. These changes could be either linear or non-linear. These relationships were observed in the present study.

The rats that had been exposed prenatally to the complex-sequenced pattern showed indications of increase ''sensitivity'' to novel stimuli. These rats showed greater conditioned taste aversion and showed more conditioned freezing responses when re-exposed to a setting that had been paired with aversion. The effect sizes of the treatments for both behaviours were about 15%. Whereas conditioned taste aversion is associated with specific gustatory stimuli and subsequent delayed sickness conditioned fear is associated with a diffuse spatial context and the immediate delivery of discrete negative stimuli.

Both paradigms involved a single pairing of a neutral stimulus with a powerful unconditioned stimulus. These results indicate that the rats exposed to the pattern (complex sequenced) designed to affect neuronal development (St-Pierre and Persinger, 2003) might be more able to avoid negative stimuli in their environments. Such enhanced vigilance and ''neophobia'' with the capacity to form associations with subtle stimuli (McKay and Persinger, 2005) may explain the consistently poorer acquisition and memory for the radial arm maze displayed by rats that had been exposed to this particular complex patterned field (McKay et al., 2003).

There was also a consistent intensity-dependence for both types of learning, regardless of the pattern of the field during the prenatal development. The rats, males and females, that had been exposed to the medium (90–580 nT) intensity prenatally showed less CTA than the other groups. However, other rats that had been exposed to this same intensity range, which is also equivalent to between 0.9 and 1.8 mG, showed greater conditioned fear.

The apparent contradiction between the direction of the effect for the CTA and the conditioned fear may be due to the reasons for the less intense CTA. The rats exposed to the medium intensity magnetic fields prenatally also drank less sucrose during the pairing. This could indicate they displayed relatively more ''bait shyness'' or ''neophobia'' even before pairing. If this explanation were valid, then this ''hypersensitivity'' would have allowed a more powerful association between the subtle contexts and the electric shocks associated with the training for conditioned fear. As a result, when the rats were tested, the context was a more powerful conditioned stimulus and the rats showed more conditioned fear.

The mechanisms by which magnetic fields in general affect semiconductive biological systems has been long assumed to be related to some form of induction of

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current (Persinger, 1974; Valentinuzzi, 1961). As reported by Adey (1990), the typical cell membrane resistances are in the range of 3,000–100,000 ohm/cm-squared while the extracelluar fluid's specific resistance is only about 50 ohm/cm-sq. Although extracelluar space occupies only 10% of the volume of brain space, it carries about 90% of any imposed current.

Assume: (1) the potential difference V, generated by the 10^{-7} T fields (100 nT; 1 mG range) changing at 10^{-4} s in a .01 m space would be in the microV range; and (2) the resistance of extracelluar fluid is about 50 ohm/cm-sq, the induced current would be within the nanoAmp range. As indicated by Becker (1965), even d.c. current in the order of 1 nA/cm is sufficient to affect cell growth presumably through processes coupled to cell-to-cell adhesion or electrostatic patterns of migration (Persinger and Robb, 1976). As predicted by Adey (1980), the effect is nonlinear and occurs within specific "windows" of intensity and frequency.

Regardless of the potential mechanisms, known or still unknown (Liboff, 1997), the results of the present study strongly indicate that long-term changes occur in the brains and bodies of rats exposed to complex magnetic fields whose intensities are well within the range of those encountered by the developing fetus within many modern habitats. It may be relevant that perinatal exposures to 0.5 Hz rotating magnetic fields whose strengths were about 10–100 times the medium intensities employed in this study have been shown to "demasculinize" the neuronal density in the medial preoptic nucleus of male rats (Mulligan and Persinger, 1998).

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